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# Association of peripheral arterial disease with periodontal disease: analysis of inflammatory cytokines and an acute phase protein in gingival crevicular fluid and serum

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*Background and Objective:* Inflammation is a common feature of both peripheral arterial disease (PAD) and periodontal disease. The aim of this study was to evaluate the relationship between PAD and periodontal disease by examining the levels of inflammatory cytokines (pentraxin 3 and interleukin 1β) and high sensitive C-reactive protein from gingival crevicular fluid and serum.

*Material and Methods:* A total of 60 patients were included in this cross-sectional study. Patients were divided into two groups based on ankle–brachial index values: with PAD (test group) and non-PAD (control group). Demographic evaluations, clinical periodontal examinations and biochemical analysis for pentraxin 3, interleukin 1β and high sensitive C-reactive protein were performed to compare the two groups.

*Results:* There were no significant differences with respect to gender, age, body mass index, or smoking history (duration, amount) between the two groups ($p > 0.05$). There were no significant differences between the two groups in terms of clinical periodontal parameters ($p > 0.05$). Neither gingival crevicular fluid nor serum levels of the cytokines showed differences between the two groups. Logistic regression analysis revealed that, after adjusting for confounding factors (age, gender, diabetes, hypertension and body mass index), periodontitis raised the odds ratio for having PAD to 5.842 (95% confidence interval: 1.558–21.909).

*Conclusion:* Although there were no significant differences with respect to clinical periodontal parameters and biochemical analyses between the study group and control, periodontitis did raise the odds ratio for having PAD. To clarify this possible relationship, future prospective studies are needed.

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*Key finding*: Periodontitis raised the odds ratio for having PAD to 5.842. No significant differences were observed between the two groups (PAD and non-PAD) in terms of serum or gingival crevicular fluid IL-1β, PTX-3 or hs-CRP levels.

Key words: cytokines; gingiva crevice fluid; periodontal disease; periodontal–systemic disease interactions

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Periodontitis is characterized by the destruction of tooth-supporting structures and leads to tooth loss and common oral health problems worldwide (1). Its association with systemic diseases has been investigated previously (2–4). The World Health Organization has stated that oral diseases, including periodontal disease, are serious and essential parts of general health (5). A new branch of periodontology, defined as "medical periodontology," has been proposed by Williams and Offenbacher, that emphasizes the bidirectional relationship between periodontal disease and systemic conditions (6). Atherosclerotic disease is the leading cause of deaths worldwide (7). Peripheral artery disease (PAD), which is generally associated with atherosclerosis, leads to lumen reduction of peripheral arteries, and its most common symptom is intermittent claudication (8). Systemic hyperinflammation plays an essential role in the onset and progression of PAD (9) and is associated with a two- to sixfold increase in the risk of cardiovascular mortality (10). Most studies have focused on the relationship between periodontal disease and cardiovascular disease (CVD) (11). To date, few studies have investigated the relationship between PAD and periodontitis (8,12–15).

Many inflammatory markers have been shown to be associated with the progression of CVD, cerebrovascular disease and PAD (16–18). Elevated levels of high sensitive C-reactive protein (hs-CRP) are a known risk factor for PAD (19). Pentraxin 3 (PTX-3), an inflammatory marker that has been the focus of a more recent study, has been investigated as a possible link between PAD and periodontitis (20). Interleukin (IL)-1β is a predictive marker for inflammatory diseases and stimulates the secretion of PTX-3 in neutrophils (21).

The aim of the present study was to evaluate the relationship between PAD and periodontal disease by examining the levels of the inflammatory cytokines (PTX-3, and IL-1β) and hs-CRP, in gingival crevicular fluid and serum. To the best of our knowledge, this was the first study that evaluated the association between periodontal disease and PAD in terms of gingival crevicular fluid cytokine and acute phase protein levels. Owing to the site-specific nature of periodontal disease, we assumed that gingival crevicular fluid could provide results with greater objectivity than those obtained with serum. In addition, it was the first study that examined serum and gingival crevicular fluid levels of PTX-3 in patients with PAD.

## Material and methods

### Study population

This cross-sectional study was conducted in full accordance with the applicable ethical principles, including the World Medical Association Declaration of Helsinki, and was independently reviewed and approved by the Ethical Committee of the Faculty of Dentistry, Erciyes University. Between September 2014 and February 2015, 84 patients were referred from the Department of Cardiovascular Surgery at Erciyes University to the Department of Periodontology for periodontal examination. Twenty-four patients were excluded from the study if they had undergone periodontal treatment within the last 3 mo; had used antibiotics within the previous 3 mo; had fewer than 10 teeth (excluding the third molar); pregnancy or lactation; or a comorbidity such as cancer, end-stage kidney disease or active rheumatic disease.

### Clinical periodontal examination

All participants were assessed clinically and radiographically for oral health. The clinical examination was performed by a single examiner (M.U.C.). Full-mouth clinical periodontal measurements were recorded at six sites (mesio-buccal, mid-buccal, disto-buccal, medio-lingual, mid-lingual and disto-lingual) per tooth, and included a plaque index (22), gingival index (23), bleeding on probing (BOP), probing depth and clinical attachment loss. The percentage of the BOP was calculated by dividing the bleeding sites by the total sites examined for each subject. Clinical attachment loss is the distance from the cemento-enamel junction in an apical direction to the base of the pocket/sulcus. A periodontal probe (Hu-Freddy, Chicago, IL USA) with Williams markings was used for the periodontal measurements. The decay-missing-filling index was also recorded. Chronic periodontitis was defined as the presence of at least five teeth with one or more sites with a probing depth of $\geq 5$ mm, a clinical attachment loss of $\geq 2$ mm, the presence of BOP and 30% radiographic bone loss (24,25). Chronic periodontitis is also characterized as localized if 30% of sites are involved and generalized if $> 30\%$ of sites are involved (25).

To estimate the reliability of the measurements during the study period, six randomly selected patients were referred to the periodontology clinic for re-evaluation. Probing depth and clinical attachment loss were evaluated, and intra-examiner reproducibility was determined as $> 0.92$ for both parameters.

### Clinical evaluation of peripheral arterial disease

The ankle–brachial index (ABI) is defined as the ratio of the blood pressure in the lower legs to the blood pressure in the arms (26). A Dopplex ABIlity-Automatic Ankle Brachial Index System (Huntleigh Healthcare Limited, Bedfordshire, UK) was used to measure the ABI for all study participants. Before the measurements, patients were rested for a minimum of 10 min, and all measurements were performed with patients in a supine position by a single examiner (Y.A.). Both the right and left arms and legs of each participant were analyzed, and the lower of the two resulting ABI values was recorded. Patients with ABI values of $\leq 0.90$ were diagnosed as having PAD (the test group), and those with ABI values of $> 1.00$ were defined as not having PAD (non-PAD, the control group). In addition to the ABI, a modified version of the Rose Questionnaire (27) and the PAD assessment scale, which

includes questions about risk factors (smoking, alcohol consumption, obesity, etc.), and examination findings (blood pressure, electrocardiogram) were also used. Biochemical analysis was also carried out including fasting glucose, total cholesterol, low-density lipoprotein and high-density lipoprotein (HDL).

Finally, study participants were divided into two groups, consisting of those with PAD ($n = 40$) and those without PAD ($n = 20$).

### Collection of samples

*Gingival crevicular fluid sampling*—The gingival crevicular fluid samples were collected on the day after clinical periodontal measurements were made with the filter paper. Patients were instructed not to consume food or liquids (except water) before the sampling procedure. The sampling area was dried and isolated with cotton rolls to prevent any contamination with saliva. A curette was gently used for supragingival plaque removal, avoiding any gingival irritation. The samples were taken at two sites on one incisor, premolar and molar tooth (a total of six samples) from each patient. In non-periodontitis patients, the mesio-buccal and disto-palatal sites of the teeth were used for sampling. In patients with periodontitis, the two deepest pockets of the same teeth served for sampling. A sterile paper strip (Oraflow, New York, NY, USA) was held for 30 s within the sulcus or pocket as per the orifice method of Rüdin *et al*. (28). In cases of visible blood and salivary contamination, the samples were discarded, and new samples were obtained. The gingival crevicular fluid volumes were measured with a calibrated device (Periotron 8000; Oraflow). All six strips for each participant were pooled in a sterile Eppendorf tube (Eppendorf, New York, USA) and stored at −80°C until the day of laboratory analysis.

*Blood sampling*—Venous blood was collected from the antecubital vein by a standard venipuncture method, and serum was separated from blood by centrifugation at 1500 ***g*** for 10 min. The resulting serum sample from each participant was divided among three microcentrifuge tubes (Eppendorf) and stored at −80°C until analysis.

### Laboratory assessment

All laboratory assessments were performed by enzyme-linked immunosorbent assay (ELISA) by the same examiner (E.S.). For each cytokine, a specific ELISA kit (Sunred, Shanghai, China) was used according to the manufacturer's instructions. For gingival crevicular fluid analyses, samples from the pooled strips were eluted in 500 μL of 0.01 M PBS, pH 7.4. Eluates (40 μL) and 10 μL primary antibodies against the analyte were added into each sample well. Total human IL-1β, human PTX-3 and human hs-CRP amounts were determined in picograms, nanograms and milligrams, respectively. The coefficient of variation for human IL-1β, human PTX-3 and human hs-CRP was 4.07%, 6.02% and 7.85%, respectively. For each cytokine, a specific ELISA kit was used as per the manufacturer's instructions. For gingival crevicular fluid analyses, samples from the pooled strips were eluted in 500 μL of 0.01 M PBS, pH 7.4. Eluates (40 μL) and 10 μL primary antibodies against the analyte were added into each sample well. Total human IL-1β, human PTX-3 and human hs-CRP amounts were determined in picograms, nanograms and milligrams, respectively. The coefficient of variation for human IL-1β, human PTX-3 and human hs-CRP was 4.07%, 6.02% and 7.85%, respectively. Collected serum sample assessments were performed by double-antibody sandwich ELISA as gingival crevicular fluid analysis. Samples (40 μL) and 10 μL primary antibodies against the analyte were added into each sample well. Total human IL-1β, human PTX-3 and human hs-CRP amounts were determined in picograms, nanograms and milligrams, respectively. The coefficient of variation for human IL-1β, human PTX-3 and human hs-CRP was 5.67%, 4.36% and 6.23%, respectively. All laboratory assessments were performed by ELISA by the same examiner (E.S.).

### Statistical analysis

The sample size was calculated taking into consideration the findings (relative to the mean difference and SD between the serum CRP levels of the study groups) of the study by Soto-Barreras *et al*. (8). Fifteen participants in each study group would be necessary to provide 80% power to detect a difference at the 0.05 significance level.

Data were analyzed using the IBM SPSS statistics 22 program (IBM, SPSS Statistics 21, Chicago, USA). The Shapiro–Wilk test was used to test the normality of the data whereas the Mann–Whitney *U*-test and Student's *t*-test were used to analyze non-parametric and parametric data, respectively. Intergroup comparisons of categorical data were made with a chi-squared analysis. To compare cytokine levels between two groups, confounding factors such as age, gender, diabetes, hypertension and body mass index (BMI) were adjusted for with an analysis of covariance (ANCOVA). The parametric data were log transformed before the ANCOVA analysis. Binary logistic regression analysis was used to estimate the odds ratio of periodontitis for PAD. Confounding factors were also adjusted for in this analysis. The level of significance was set to $p < 0.05$.

## Results

Demographic data for the two groups of participants are shown in Table 1. There were no significant differences with respect to gender, age, BMI or smoking history (duration, amount) between the two groups ($p > 0.05$). The mean age ± SD was 57.4 ± 11 years and 60.4 ± 9 years for the non-PAD (control) and PAD (test) group, respectively; 90% and 80% of the participants were male for the control and test group, respectively. No significant differences were observed between the groups in terms of their

medical history, including diabetes mellitus, hypertension, hyperlipidemia, cerebrovascular disease or CVD (Table 2).

There were no significant differences between the two groups in terms of clinical periodontal parameters (plaque index, gingival index, BOP, probing depth, clinical attachment loss; Table 3). However, whereas there were significantly more patients with gingivitis in the control group, the majority of patients in the PAD group had localized and generalized chronic periodontitis ($p < 0.05$) (Tables 3 and 4). The number of sites with $\geq 5$ mm probing depth was significantly higher in the PAD group as compared with the control group (Table 3). No significant differences were observed for decay-missing-filling index scores between these two groups.

No significant differences with respect to biochemical parameters, including fasting glucose, total cholesterol and low-density lipoprotein, were found except for HDL between these two groups (Table 5). Cytokine (IL-1β, PTX-3) and acute phase protein (hs-CRP) levels from both gingival crevicular fluid and serum were not different between the two groups (Table 6).

Logistic regression analysis revealed that after adjusting for confounding factors (age, gender, diabetes, hypertension and BMI), periodontitis raised the odds ratio for having PAD to 5.842 (95% confidence interval [95% CI]: 1.558–21.909) (Table 7).

## Discussion

There are several risk factors for PAD including aging, diabetes and smoking (29). The incidence and prevalence of PAD increases with increasing age, with a prevalence ranging from 2.5% for individuals aged 50–59 years to 14.5% for those > 70 years (29). The fact that 90% of the PAD group was male may be considered as corroborative evidence when the findings of Norgren *et al.* (29) were taken into account who reported that the prevalence of PAD is greater in men.

Systemic diseases, including diabetes mellitus, hypertension, dyslipidemia and chronic renal disease, are important risk factors for PAD (29). Patients with diabetes mellitus and symptoms of claudication have a 20% amputation risk and their 5 year risk of death is 50% (30). Kannel and McGee (31) reported that the PAD risk was 2.5–4-fold higher in patients with hypertension. Kannel and Shurtleff (32) documented that > 7 mmol/L fasting cholesterol was associated with the incidence of episodic claudication, and HDL and total cholesterol were defined as the best predictors by the authors. All of the risk factors mentioned above were not significantly different between the two groups in our study. This situation, allowed us to examine the association between periodontal disease and PAD.

Although many studies investigated the relationship between periodontal disease and cerebrovascular disease and CVD (11,33), only several have examined the relationship between periodontal disease and PAD (8,12–15). Mendez *et al.* (13) hypothesized that periodontal disease is an independent risk factor for PAD taking into consideration the similar pathophysiological mechanism of both diseases. The authors concluded that subjects with clinically significant periodontal disease had a 2.27-fold increase in the risk of developing PAD. Hung *et al.* (14) conducted a prospective cohort study ($n = 342$) and reported that periodontal disease was associated with a relative risk of 1.41 (95% CI: 1.12–1.77) for developing PAD during a 12 year follow-up. The retrospective study by Lu *et al.* (15) on 3585 subjects revealed that > 3 mm of attachment loss was associated with a > 2-fold increase in the risk of PAD after adjusting for other risk factors. Chen *et al.* (12) investigated the association between periodontitis and

*Table 1.* Demographic data of the study participants

| Characteristic | Group | | *p* value |
|---|---|---|---|
| | Non-PAD group (Control) | PAD group (Test) | |
| Gender | | | |
| Female (*n*) (%) | 2 (10%) | 8 (20%) | 0.471 |
| Male (*n*) (%) | 18 (90%) | 32 (80%) | |
| Age mean ± SD | 57.40 ± 11.16 | 60.45 ± 9.94 | 0.287 |
| BMI | | | |
| Median | 27.45 | 25.97 | 0.193 |
| Min–max | 22.2–34.94 | 20.0–39.94 | |
| Smoking habits | | | |
| Current smoker | 8 (40%) | 20 (50%) | 0.750 |
| Former smoker | 4 (20%) | 6 (15%) | |
| Non-smoker | 8 (40%) | 14 (35%) | |
| Alcohol habits | | | |
| Current user | 2 (10%) | 2 (5%) | 0.732 |
| Former user | – | 1 (2.5%) | |
| None | 18 (90%) | 37 (92.5%) | |

*Table 2.* Systemic diseases or conditions of the study participants

| Disease or condition | Group | | *p* value |
|---|---|---|---|
| | Non-PAD group (Control) | PAD group (Test) | |
| Diabetes mellitus (*n*) (%) | 7 (35%) | 12 (30%) | 0.772 |
| Hypertension (*n*) (%) | 6 (30%) | 15 (37.5%) | 0.775 |
| Hyperlipidemia (*n*) (%) | 3 (15%) | 9 (22.5%) | 0.734 |
| Cerebrovascular disease (*n*) (%) | 1 (5%) | 3 (7.5%) | 1.00 |
| Cardiovascular disease (*n*) (%) | 19 (95%) | 37 (92.5%) | 0.734 |

PAD and examined the localization of periodontopathic bacteria in atherosclerotic specimens. Serum IgG titers against bacteria and serum inflammatory cytokines including IL-6, tumor necrosis factor-α and IL-1β were also examined. Their findings suggested that periodontitis is associated with an increased risk of PAD (12). This association could result from the increased concentration of serum inflammatory cytokines in patients with periodontitis. To the best our knowledge, the most recent study that focused on this issue found that a positive relationship exists between periodontal disease and PAD (8). Three of the above-mentioned studies (13–15) were all epidemiological studies with large populations. Consequently, there are methodological differences between those studies and the present study. Mendez *et al.* (13) evaluated periodontal health status based solely on radiographic bone levels. Lu *et al.* (15) made a diagnosis of periodontitis based on only the presence of ≥ 3 mm attachment loss. Lastly, Hung *et al.* (14) used a questionnaire-based evaluation to diagnose their study participants. Those differences make it impossible for us to compare their findings with the findings from this study.

A binary logistic regression multivariate analysis model of the data here showed a positive association between periodontitis and PAD (adjusted for age, smoking, diabetes mellitus, hypertension and BMI). Periodontitis raised the odds ratio for having PAD to 5.84. This finding is in agreement with the findings of Chen *et al.* (12) (odds ratio, 5.45; 95% CI, 1.57–18.89) and Soto-Barreras *et al.* (8) (odds ratio, 8.18; 95% CI, 1.21–35.23). Different mechanisms may be responsible for the increased risk of PAD in patients with periodontitis: (i) bacteria may damage endothelial tissues (8), which may be defined as a "direct vascular injury"; (ii) gram-negative bacteria produce lipopolysaccharide, which leads to chronic activation of circulating monocytes and the expression of different molecules such as the scavenger receptor and CD68/macrosialin, which play a role in atherogenesis; (iii) serum antibodies against heat shock proteins 65/60 may have a role in atherosclerosis by cytotoxic activity (13) and, finally (iv) increased proinflammatory susceptibility may serve as an alternative pathway for developing atherosclerosis and periodontitis (34).

IL-1β is an important cytokine that plays a role in the progression of periodontal disease and in the initiation of the inflammation cascade (35). IL-1β levels are higher in patients with periodontitis when compared with patients with a healthy periodontium (35). IL-1β also has effects on the cell types that make up atherosclerotic lesions (36). Chen *et al.* (12) suggested that the association between periodontitis and increased risk of PAD might be explained by the increased concentration of serum cytokines in patients with periodontitis. Our findings seem to corroborate the suggestion of Chen *et al.* (12), but this finding should be interpreted cautiously, as age and gender have significant effects on serum cytokine levels (37) and thus should be adjusted for when analyzing these data.

PTX-3 is a novel acute phase protein that is a diagnostic and prognostic marker for periodontal disease and atherosclerotic disease (38). Leukocytes, dendritic cells, monocytes and macrophages can express PTX-3 in response to primary inflammatory signals (39). Rolph *et al.* (40) documented PTX-3 expression in advanced human atherosclerotic lesions and

*Table 3.* Periodontal parameters and DMFT scores of the study participants

| Parameter | Group | | *p* value |
|---|---|---|---|
| | Non-PAD group (control) | PAD group (test) | |
| PI | | | |
| Median | 1.91 | 2.0 | 0.456 |
| Min–max | 0.85–2.53 | 0.86–2.33 | |
| GI | | | |
| Median | 1.81 | 1.96 | 0.600 |
| Min–max | 1.01–2.16 | 1.0–2.40 | |
| BOP | | | |
| Median | 3.41 | 5.18 | 0.058 |
| Min–max | 0–19.4 | 0–17.5 | |
| PD | | | |
| Median | 2.1 | 2.5 | 0.072 |
| Min–max | 1.36–4.75 | 1.46–4.54 | |
| CAL | | | |
| Mean ± SD | 3.82 ± 0.69 | 3.88 ± 0.91 | 0.642 |
| PD severity | | | |
| Gingivitis (*n*) (%) | 12 | 10 | 0.031 |
| Localized chronic periodontitis (*n*) (%) | 5 | 21 | |
| Generalized chronic periodontitis (*n*) (%) | 3 | 9 | |
| Sites with ≥ 5 mm PD | | | |
| Median | 0 | 6 | 0.019 |
| Min–max | 0–31 | 0–44 | |
| DMFT scores | | | |
| Mean ± SD | 12.65 ± 6.30 | 14.60 ± 5.46 | 0.221 |

BOP, bleeding on probing; CAL, clinical attachment loss; DMFT, decay-missing-filling index; GI, gingival index; PAD, peripheral arterial disease; PD, probing depth; PI, plaque index.

*Table 4.* Contingency table of the relationship of PAD positive/negative and PD positive/negative

| | Non-PAD (*n*) | PAD group(*n*) |
|---|---|---|
| Periodontitis | 8 (40%) | 30 (75%) |
| Non-periodontitis | 12 (60%) | 10 (25%) |
| Total % | 100 | 100 |

PAD, peripheral arterial disease; PD, periodontal disease.

suggested that it may contribute to the pathogenesis of atherosclerosis. Pradeep *et al*. (41) suggested that PTX-3 is a good indicator for periodontal tissue destruction, and its association with periodontal clinical parameters has been shown. As PTX-3 is secreted locally at the inflammatory gingival tissues, its levels in the gingival crevicular fluid may better correlate with the disease severity (39). Zhou *et al*. (20) reported that a high level of PTX-3 is a potent risk factor for PAD when compared with CRP. It is believed that PTX-3 leads to neutrophil accumulation, which results in atherosclerotic lesions (40). Our findings did not concur with these previous findings, as we found no statistical difference in serum or gingival crevicular fluid PTX-3 levels between the PAD and non-PAD groups. We note, however, that this comparison may be flawed, as Zhou *et al*. (20) performed their study on hemodialysis patients and did not adjust for age or gender during the statistical analysis of the data.

The association between CRP levels and an increase in the severity of PAD is true not only for coronary and cerebral vessels but also for peripheral circulation (42). Soto-Barreras *et al*. (8) and Ridker *et al*. (43) reported that CRP levels increase in patients with PAD. Moreover, inflammation associated with chronic infections such as periodontitis may lead to an increase in CRP levels (44) and an association may exist between the measured CRP levels and atherosclerotic disease (8,43). Tüter *et al*. (45) reported a significant increase in serum hs-CRP levels but no difference in gingival crevicular fluid hs-CRP levels between patients with chronic periodontitis with coronary artery disease and healthy control patients. In the present study, we did not find any significant differences between the two groups in terms of CRP levels in either serum or gingival crevicular fluid. This result did not concur with the findings of Soto-Barreras *et al*. (8) and Ridker *et al*. (43). Although the number of periodontitis patients and the number of sites per patient with > 5 mm probing depth were higher in the PAD group as compared with the non-PAD group, the presence of periodontal disease should not be evaluated as the sole factor affecting systemic inflammation. High blood pressure, chronic fatigue, alcohol consumption, high triglyceride levels, insulin resistance, estrogen medication, diabetes mellitus, a protein-rich diet, sleep disturbance, smoking and depression can all increase serum CRP levels (44,46).

The above-mentioned surprising findings opposing the well-known positive relationship between inflammatory cytokines and PAD may be explained by the heterogeneity of the study groups. Authors were not able to form homogeneous study groups

*Table 5*. Biochemical parameters of the study participants

| | Group | | |
|---|---|---|---|
| Parameter | Non-PAD group (control) | PAD group (test) | *p* value |
| Fasting glucose | | | |
| Median | 92 | 92.5 | 0.802 |
| Min–max | 56–166 | 44–451 | |
| Total cholesterol | | | |
| Median | 195 | 173.5 | 0.089 |
| Min–max | 142–306 | 111–257 | |
| High-density lipoprotein | | | |
| Median | 43 | 38.5 | 0.023 |
| Min–max | 24–89 | 17–54 | |
| Low-density lipoprotein | | | |
| Median | 116 | 103 | 0.124 |
| Min–max | 56–211 | 46–173 | |

*Table 6*. Cytokine and acute phase protein levels of the study participants

| | Group | | |
|---|---|---|---|
| | Non-PAD group (control) | PAD group (test) | *p* value |
| GCF | | | |
| IL-1 beta | 1138.6 ± 245 | 1098.1 ± 242 | 0.278 |
| PTX-3 | 2.33 ± 0.44 | 2.68 ± 0.63 | 0.279 |
| hs-CRP | 5.96 ± 0.89 | 5.81 ± 1.0 | 0.620 |
| Serum | | | |
| IL-1 beta (Log) | 3.31 ± 0.4 | 3.18 ± 0.3 | 0.099 |
| PTX-3 (Log) | 0.621 ± 0.1 | 0.485 ± 0.1 | 0.060 |
| hs-CRP (Log) | 0.924 ± 0.2 | 0.920 ± 0.1 | 0.529 |

GCF, gingival crevicular fluid; hs-CRP, high sensitive C-reactive protein; IL, interleukin; PAD, peripheral arterial disease; PTX-3, pentraxin 3.

*Table 7*. Binary logistic regression

| Variable | Odds ratio | 95% confidence interval | *p* value |
|---|---|---|---|
| Periodontitis | 5.842 | 1.558–21.909 | 0.009 |
| Smoking habits | | | |
| Former smokers | 1.411 | 0.303–6.574 | 0.661 |
| Current smokers | 1.079 | 0.170–6.872 | |
| Age | 1.042 | 0.975–1.113 | 0.228 |
| Gender (male) | 8.383 | 0.874–80.389 | 0.064 |
| Diabetes mellitus | 0.876 | 0.232–3.472 | 0.876 |
| Hypertension | 1.021 | 0.238–4.367 | 0.875 |
| Body mass index | 0.912 | 0.787–1.059 | 0.227 |

due to the advanced age of the participants who applied to the Department of Cardiovascular Surgery (Erciyes University, Kayseri, Turkey). In other words, patients taking various medications for different systemic problems did play an important part in the study population heterogeneity. In addition, smoking and alcohol consumption status of the participants could have also played a role in this issue, as, particularly the former is well known to modify the production of cytokines or inflammatory mediators (47,48).

To the best of our knowledge, this was the first study that evaluated the association between periodontal disease and PAD in terms of gingival crevicular fluid cytokine and acute phase protein levels. In addition, it was the first to examine serum and gingival crevicular fluid PTX-3 levels in patients with PAD. The main reason the authors used gingival crevicular fluid samples in addition to serum samples was that whereas serum reflects systemic inflammation, gingival crevicular fluid reflects local inflammation of periodontal tissues.

A major limitation of the present study was the absence of information regarding the use of anti-inflammatory drugs by the patients with systemic diseases and their possible effects on masking the severity of inflammation in patients with periodontitis. This limitation might have been overcome by rigid patient selection criteria; however, this was difficult because of the high number of elderly patients in the study population and extensive variability in their use of drugs for systemic problems. This heterogeneity in the study population also precluded adjustments for systemic factors (except for diabetes mellitus and hypertension) in the statistical analysis.

## Conclusions

Within the limitations of this study, there were no significant differences between the two groups in terms of clinical periodontal parameters. However, the striking finding was that periodontitis raised the odds ratio for having PAD to 5.842. No significant differences were observed between the two groups in terms of serum or gingival crevicular fluid IL-1β, PTX-3 or hs-CRP levels. Future prospective studies are needed to clarify the association between periodontitis and PAD.

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## Conflicts of interest

The authors declare that there is no conflict of interest concerning the contents of the study.

## References

1. Petersen PE, Baehni PC. Periodontal health and global public health. *Periodontol 2000* 2012;**60**:7–14.
2. Oppermann RV, Weidlich P, Musskopf ML. Periodontal disease and systemic complications. *Braz Oral Res* 2012;**26**:39–47.
3. Garcia RI, Henshaw MM, Krall EA. Relationship between periodontal disease and systemic health. *Periodontol 2000* 2001;**25**:21–36.
4. Taşdemir Z, Alkan BA. Knowledge of medical doctors in Turkey about the relationship between periodontal disease and systemic health. *Braz Oral Res* 2015;**29**:1–8.
5. World Health Organization. *The Liverpool Declaration: Promoting Oral Health in the 21st Century*. A call for action. Geneva: WHO, 2005:2 p.
6. Williams RC, Offenbacher S. Periodontal medicine: the emergence of a new branch of periodontology. *Periodontol 2000* 2000;**23**:9–12.
7. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med* 2011;**17**:1410–1422.
8. Soto-Barreras U, Olvera-Rubio JO, Loyola-Rodriquez JP *et al*. Peripheral arterial disease associated with caries and periodontal disease. *J Periodontol* 2013;**84**:486–494.
9. Khawaja JF, Kullo JI. Novel markers of peripheral arterial disease. *Vasc Med* 2009;**14**:381–392.
10. Hirsch AT, Crigui MH, Treat-Jacobson D *et al*. Peripheral arterial disease detection, awareness, and treatment in primary care. *JAMA* 2001;**286**:1317–1324.
11. Blaizot A, Vergnes JN, Nuwwareh S, Amar J, Sixou M. Periodontal disease and cardiovascular events: meta-analysis of observational studies. *Int Dent J* 2009;**59**:197–209.
12. Chen YW, Umeda M, Nagasawa T *et al*. Periodontitis may increase the risk of peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2008;**35**:153–158.
13. Mendez MV, Scott T, LaMorte W, Vokonas P, Menzoian JO, Garcia R. An association between periodontal disease and peripheral vascular disease. *Am J Surg* 1998;**176**:153–157.
14. Hung HC, Willett W, Merchant A, Rosner BA, Ascherio A, Joshipura KJ. Oral health and peripheral arterial disease. *Circulation* 2003;**107**:1152–1157.
15. Lu B, Parker D, Eaton CB. Relationship of periodontal attachment loss to peripheral vascular disease: an analysis of NHANES 1999–2002 data. *Atherosclerosis* 2008;**200**:199–205.
16. Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular disease: a focus on endothelial responses to inflammation. *Clin Sci (Lond)* 2005;**108**:205–213.
17. Botti C, Maione C, Dogliotti G *et al*. Circulating cytokines present in the serum of peripheral arterial disease patients induce endothelial dysfunction. *J Biol Regul Homeost Agents* 2012;**26**:67–79.
18. Moreno VP, Subira D, Meseguer E, Llamas P. IL-6 as a biomarker of ischemic cerebrovascular disease. *Biomark Med* 2008;**2**:125–136.
19. Shankar A, Li J, Nieto FJ, Klein BE, Klein R. Association between C-reactive protein level and peripheral arterial disease among US adults without cardiovascular disease, diabetes, or hypertension. *Am Heart J* 2007;**154**:495–501.
20. Zhou Y, Zhang J, Zhu M, Lu R, Wang Y, Ni Z. Plasma pentraxin 3 is closely associated with peripheral arterial disease in hemodialysis patients and predicts clinical outcome: a 6-year follow-up. *Blood Purif* 2015;**39**:266–273.
21. Inoue K, Kodama T, Daida H. Pentraxin-3: a novel biomarker for inflammatory cardiovascular disease. *Int J Vasc Med* 2012;**2012**:657025. doi: 10.1155/2012/657025.
22. Silness J, Löe H. Periodontal disease in pregnancy. 3. Response to local

treatment. *Acta Odontol Scand* 1966;**24**:747–759.
23. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;**21**:533–551.
24. Savage A, Eaton KA, Moles DR, Needleman I. A systemic review of definitions of periodontitis and methods that have been used to identify this disease. *J Clin Periodontol* 2009;**36**:458–467.
25. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;**4**:1–6.
26. Aboyans V, Criqui MH, Abraham P *et al*. Measurement and interpretation of the Ankle-Brachial Index. *Circulation* 2012;**126**:2890–2909.
27. Criqui MH, Denenberg JO, Bird CE *et al*. The correlation between symptoms and non-invasive test results in patients referred for peripheral arterial disease testing. *Vasc Med* 1996;**1**:65–71.
28. Rüdin HJ, Overdiek HF, Rateitschak KH. Correlation between sulcus fluid rate and clinical and histological inflammation of the marginal gingiva. *Helv Odontol Acta* 1970;**14**:21–26.
29. Norgren L, Hiatt WR, Dormandy DA, Nehler MR, Harris KA, Fowkes FG. Inter-society consensus for the management of peripheral arterial disease (TASC II). *J Vasc Surg* 2007;**45**:5–67.
30. Wattanakit K, Folsom AR, Selvin E *et al*. Risk factors for peripheral arterial disease incidence in person with diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis* 2005;**180**:389–397.
31. Kannel WB, McGee DL. Update on some epidemiologic features of intermittent claudication: the Framingham study. *J Am Geriatr Soc* 1985;**33**:13–18.
32. Kannel WB, Shurtleff D. The Framingham study. Cigarettes and development of intermittent claudication. *Geriatrics* 1973;**28**:61–68.
33. Leira Y, Blanco M, Blanco J, Castillo J. Association between periodontal disease and cerebrovascular disease. A review of the literature. *Rev Neurol* 2015;**61**:29–38.
34. Jimenez M, Krall EA, Garcia RI, Vokonas PS, Dietrich T. Periodontitis and incidence of cerebrovascular disease in men. *Ann Neurol* 2009;**66**:505–512.
35. Tsai CC, Ho YP, Chen CC. Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adults periodontitis. *J Periodontol* 1995;**66**:852–859.
36. Kirii H, Niwa T, Yamada Y *et al*. Lack of interleukin-1 beta decreases the severity of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 2003;**23**:656–660.
37. Larsson A, Carlsson L, Gordh T, Lind AL, Thulin M, Kamali-Moghaddam M. The effects of age and gender on plasma levels of 63 cytokines. *J Immunol Methods* 2015;**425**:58–61.
38. Jenny NS, Blumenthal RS, Kronmal RA, Rotter JI, Siscovick DS, Psaty BM. Association of pentraxin 3 with cardiovascular disease: the multi-ethnic study of atherosclerosis. *J Thromb Haemost* 2014;**12**:999–1005.
39. Garlanda C, Maina V, Cotena A, Moalli F. The soluble pattern recognition receptor pentraxin-3 in innate immunity, inflammation and fertility. *J Reprod Immunol* 2009;**83**:128–133.
40. Rolph MS, Zimmer S, Bottazzi B, Garlanda C, Mantovani A, Hansson GK. Production of the long pentraxin PTX3 in advanced atherosclerotic plaques. *Arterioscler Thromb Vasc Biol* 2002;**22**: e10–e14.
41. Pradeep AR, Kathariya R, Raghavendra NM, Sharma A. Levels of pentraxin-3 in gingival crevicular fluid and plasma in periodontal health and disease. *J Periodontol* 2011;**82**:734–741.
42. De Haro J, Acin F, Medina FJ, Lopez-Quintana A, March JR. Relationship between the plasma concentration of C-reactive protein and severity of peripheral arterial disease. *Clin Med Cardiol* 2008;**3**:1–7.
43. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a) and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA* 2001;**285**:2481–2485.
44. Bansal T, Pandey A, Asthana AK. C-reactive protein (CRP) and its association with periodontal disease: a brief review. *J Clin Diagn Res* 2014;**7**:21–24.
45. Tüter G, Kurtis B, Serdar M. Evaluation of gingival crevicular fluid and serum levels of high-sensitivity C-reactive protein in chronic periodontitis patients with or without coronary artery disease. *J Periodontol* 2007;**78**:2319–2324.
46. Graziani F, Cei S, Tonetti M *et al*. Systemic inflammation following non-surgical and surgical periodontal therapy. *J Clin Periodontol* 2010;**37**:848–854.
47. Giannopoulou C, Kamma JJ, Mombelli A. Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. *J Clin Periodontol* 2003;**30**:145–153.
48. Crews FT, Bechara R, Brown LA *et al*. Cytokines and alcohol. *Alcohol Clin Exp Res* 2006;**30**:720–730.